American Journal of Pathology, Vol. 154, No. 2, February 1999

Short Communication

Microvessels from Alzheimer's Disease Brains Kill Neurons *in Vitro*

Paula Grammas,*† Pete Moore,*† and Paul H. Weigel†‡

From the Departments of Pathology and Biochemistry and Molecular Biology‡ and The Oklahoma Center for Neuroscience,† University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma*

Understanding the pathogenesis of Alzheimer's disease is of widespread interest because it is an increasingly prevalent disorder that is progressive, fatal, and currently untreatable. The dementia of Alzheimer's disease is caused by neuronal cell death. We demonstrate for the first time that blood vessels isolated from the brains of Alzheimer's disease patients can directly kill neurons *in vitro*. Either direct co-culture of Alzheimer's disease microvessels with neurons or incubation of cultured neurons with conditioned medium from microvessels results in neuronal cell death. In contrast, vessels from elderly nondemented donors are significantly ($P < 0.001$) less lethal and brain vessels from younger donors are not neurotoxic. Neuronal killing by either direct co-culture with Alzheimer's disease microvessels or conditioned medium is dose- and time-dependent. Neuronal death can occur by either apoptotic or necrotic mechanisms. The microvessel factor is neurospecific, killing primary cortical neurons, cerebellar granule neurons, and differentiated PC-12 cells, but not non-neuronal cell types or undifferentiated PC-12 cells. Appearance of the neurotoxic factor is decreased by blocking microvessel protein synthesis with cycloheximide. The neurotoxic factor is soluble and likely a protein, because its activity is heat labile and trypsin sensitive. These findings implicate a novel mechanism of vascular-mediated neuronal cell death in Alzheimer's disease. ***(Am J Pathol 1999, 154:337–342)***

Alzheimer's disease (AD) is a neurodegenerative disorder of unknown cause that affects over 4 million older Americans. The disease is characterized by dementia, amyloid-β deposition, plaques, tangles, and neuronal cell loss.[1] The familial forms of early-onset AD, which account for 2 to 7% of AD patients, are associated with genes for presenilin proteins located on chromosomes 1 and 14, and the β-amyloid precursor protein on chromosome 21.[2–4] Inheritance of the ApoE allele ϵ4 (on chromosome 19) is a risk factor for the development of late-onset AD.[5] Despite intensive research efforts, the causes of neuronal loss in the most common form of AD (sporadic AD) remain unknown.

Recent studies suggest an important role for blood vessels in the pathogenesis of AD dementia.[6–8] A 15-year longitudinal study of blood pressure and dementia supports the concept that vascular factors are involved in the development of AD. Population-based studies have linked atherosclerosis, the ApoE genotype, and prevalence of dementia. Snowdon and colleagues[8] found dementia in individuals with classic AD pathology, consisting of plaques and tangles, only when superimposed on cerebrovascular disease.

Numerous structural and functional abnormalities of the cerebromicrovasculature in AD have been identified,[9–13] including decreased microvascular density and vascular distortions such as vessel kinking, twisting, tortuosity, and looping.[11] In addition, several active functions of the blood-brain barrier, including glucose transport, are diminished in AD.[13] We have previously shown that brain microvessels isolated from human postmortem material remain viable and that many biochemical processes, especially signaling events, are amenable to study.[14–18] Microvessels from AD patients demonstrate receptor abnormalities and intracellular signal transduction defects, especially in protein kinase C and cAMP pathways.[14–18] Furthermore, AD brain vessels are oxidatively damaged, express inflammatory mediators, and over-produce nitric oxide.[18,19] Elevated vascular production of nitric oxide, a potential neurotoxin, could contribute to neuronal injury and death in AD. These data taken

Supported in part by National Institute of Neurological Disorders and Stroke, NIH Grant NS30457 (P. Grammas), Alzheimer's Association IIRG-95–158 (P. Grammas), and Presbyterian Health Foundation (P. Grammas).

Accepted for publication November 4, 1998.

Address reprint requests to Paula Grammas, University of Oklahoma Health Sciences Center, Department of Pathology, 975 N.E. Tenth Street, BRC 262, Oklahoma City, OK 73104. E-mail: paula-grammas@ouhsc.edu.

together suggest that vessels are dysfunctional in AD. The objective of this study was to determine whether blood vessels from AD patients are directly injurious to neurons in culture.

Materials and Methods

Human Microvessel Isolation

Human autopsy brain specimens were obtained approximately 6 to 11 hours postmortem and frozen at −70°C until dissection. The clinical diagnosis of primary AD was confirmed by neuropathological examination. Control samples both age-matched and younger were taken from patients without evidence of neuropathology. Microvessels were isolated from pooled temporal, parietal, and frontal cortices; filtration through a 210-μm sieve and collection on a 53-μm sieve was as previously described.[14] This procedure yields approximately 6 to 10 mg microvessel protein from 15 g of human cortex. A separate microvessel preparation was isolated from each human brain. The purity of the microvessel preparations was routinely monitored by phase-contrast microscopy.[17] Microvessels were then resuspended in Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal calf serum and 10% dimethylsulfoxide and stored in liquid nitrogen until used.

Preparation of Microvessel-Conditioned Media

Microvessels stored in liquid nitrogen were quick-thawed at 37°C and centrifuged at 2000 × *g* for 10 minutes. The vessels were washed 3 times with cold Hanks' balanced salt solution and resuspended in serum-free DMEM (2 ml) containing 1% lactalbumin hydrolysate. Microvessels were allowed to recover for 1 hour, after which they released little lactate dehydrogenase (LDH) indicating viability. The microvessels were washed by centrifugation and resuspended at a concentration of 100 μg of protein per ml in serum-free DMEM containing 1% lactalbumin hydrolysate. The microvessels were then incubated for variable times (4 to 24 hours) in a CO_2 incubator, centrifuged, and the conditioned medium was sterile-filtered through a 0.4-μm filter and used immediately.

Primary Neuronal and PC-12 Cell Cultures

Cerebral cortices and cerebella were isolated from 17-day-old fetal rats, dissociated, and seeded in polylysine-coated plates containing DMEM with 5% horse serum, as previously described.[20] After 5 days of culture, the cells were treated with 5-fluoro-deoxyuridine and maintained for 2 to 3 weeks before use. The cultures were identified as 88% neuronal by flow cytometry using an antibody to neuronal-specific enolase.

PC-12 cell (rat pheochromocytoma cells; American Type Culture Collection, Rockville, MD) cultures were maintained in RPMI 1640 media supplemented with 2 mmol/L glutamine, 10% heat-inactivated horse serum, and 5% fetal calf serum. Cells were plated into 24-well dishes at approximately 10^5 cells/well. Seven to 14 days before use, 50 ng/ml nerve growth factor was added to each well to stimulate neuronal differentiation.[21]

Determination of Neuronal Cell Death by Necrosis and Apoptosis

Cell death (necrosis) was determined by release of cytoplasmic LDH.[20] Medium (100 μl) was removed, added to a 96-well plate, the chromogenic substrate added, and the plate was incubated at room temperature for 30 minutes in darkness. The A_{490} of each well was measured to determine LDH activity. Data (percent cytotoxicity) were expressed as a percentage of total LDH released by treatment with 1% Triton X-100 added to the same well. Vascular LDH release, determined for microvessel-containing inserts placed in wells without neuronal cells, was subtracted from each co-culture point. Each point was performed in duplicate.

Cell death (apoptosis) was determined by enzyme-linked immunosorbent assay measurement of nucleosomes using a kit from Boehringer Mannheim (Indianapolis, IN). Neurons were collected by scraping, washed, incubated 30 minutes at 4°C with lysis buffer, centrifuged at 15,000 × *g* for 10 minutes at 4°C, and aliquots were transferred to a microtiter plate precoated with an antibody to histone. After washing 3 times, a second antibody to DNA conjugated to horseradish peroxidase was added and the plate was incubated 90 minutes at room temperature and washed 3 times. The peroxidase substrate was added to each well and the plate was incubated for 10 to 20 minutes. Absorbance was read at 405 nm. The amount of microvessel-mediated apoptotic neuronal cell death was expressed as a percentage of the apoptosis (100%) elicited by 10 μmol/L sodium nitroprusside, a nitric oxide releasing compound.[22]

Statistical Analysis

Unless otherwise indicated, data presented are mean ± SE. Statistical analysis between two groups was performed using the Student's *t*-test, and for comparisons among multiple samples, the analysis of variance was used.

Results

AD Microvessel-Conditioned Media or Direct Co-Culture Causes Neuronal Cell Death

We found that either media conditioned by AD microvessels or direct co-culture of AD microvessels with neurons caused neuronal cell death. Using microvessel-conditioned media, we established a quantitative and convenient assay for neurotoxicity in which cell death was linear with the amount of conditioned medium (Figure 1A). The neurotoxicity of conditioned media from AD brain microvessels, expressed as percent cell death per μl under standardized conditions, was significantly ($P < 0.001$) greater than that of conditioned media from age-matched

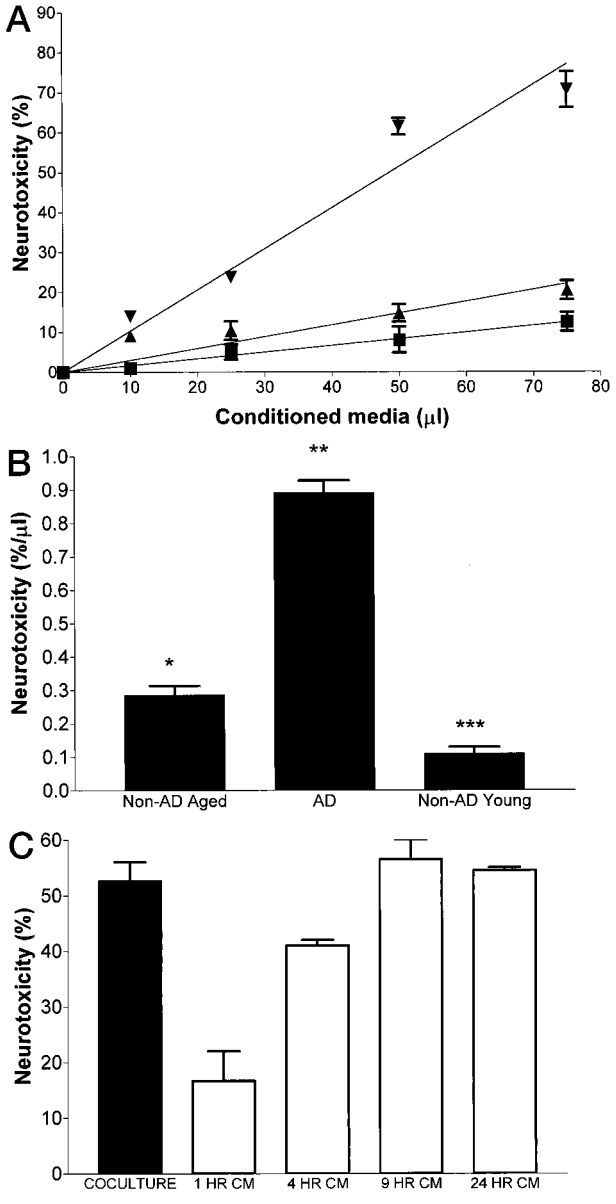

Figure 1. Conditioned medium from AD microvessels causes neuronal cell death. Conditioned medium was prepared under standardized conditions (100 μg protein/ml) by incubating brain microvessels from AD (73 ± 5.7 years, range 62 to 80 years), age-matched non-AD (71.8 ± 5.7 years, range 59 to 81 years), and non-AD young patients (43.9 ± 13.5 years, range 21 to 58 years) at 37°C for 4 hours. Different volumes of conditioned medium were placed into 24-well plates with primary cerebral cortical cultures, incubated at 37°C for 24 hours, and 100 μl of aliquots of medium were then assayed for neuronal LDH release. For **A**, each line is an individual case examined over the indicated volume range of microvessel-conditioned media: AD (▼), non-AD aged (▲), and non-AD young (■). For **B**, the mean slopes ± SE from a larger number of AD (n = 9), non-AD aged (n = 9), and non-AD young (n = 6) cases were determined. The slope is defined as the neurotoxicity of the sample, % cell death/μl conditioned medium. Slopes were determined by linear regression analysis, and had an average correlation coefficient (n = 24) of 0.96. *$P < 0.001$, significantly different from non-AD young; **$P <$ 0.001, significantly different from non-AD aged; ***$P < 0.001$, significantly different from AD. For **C**, conditioned medium from AD microvessels was collected at 1, 4, 9, and 24 hours. Primary cerebral cortical cultures were treated with AD microvessels (100 μg) in co-culture or conditioned medium (1 ml) and incubated 4 hours at 37°C. Aliquots of the culture medium (100 μl) were then removed and neuronal cell death assessed by LDH release. Data are mean ± SE of two separate experiments each performed in triplicate.

non-AD controls. The conditioned medium from microvessels of even younger (32 to 59 years) non-AD patients caused essentially no neuronal death. The results are summarized in Figure 1B for a large number of AD and control cases.

Microvessel-conditioned media, collected between 4 and 9 hours, evoked neuronal cell death comparable with that of AD microvessels in co-culture for 4 hours. The accumulation of neurotoxic factor(s) in conditioned medium plateaued after about 9 hours of exposure to microvessels and remained constant for at least 24 hours (Figure 1C). These results demonstrate that the lethal effect of AD microvessels is not dependent on a feedback loop between neurons and microvessels because microvessel-conditioned medium is also neurotoxic.

Neurotoxic Factor Is Inhibited by Cycloheximide and is Trypsin Sensitive and Heat Labile

Incubation of Alzheimer's disease microvessels with cycloheximide (10 μg/ml) inhibited appearance of the neurotoxic factor as assessed by a 60% decrease in neuronal necrosis. The factor is likely to be a protein because the neurotoxic activity is both trypsin-sensitive and heat-labile. Incubation of AD conditioned media at 55°C for 40 minutes or incubation with 0.1 mg/ml TPCK-treated trypsin for 1 hour at 37°C results in loss of neurotoxic activity 76 and 83%, respectively.

Vascular-Mediated Neuronal Cell Death Is Not Mediated by Nitric Oxide, Tumor Necrosis Factor-α (TNF-α), and Amyloid β

Although the identity of the neurotoxic factor(s) is unknown, our initial experiments have ruled out the involvement of several possible candidate molecules. Nitric oxide was not responsible for the microvessel-mediated neurotoxicity because preincubation of microvessels with the nitric oxide synthase L-1-tosylamide-2-phenylethyl chloromethyl ketone inhibitor 10 μmol/L N^GNitro-L-arginine, a concentration shown to inhibit microvascular nitric oxide production,[18] did not affect toxicity of the conditioned media (% neurotoxicity of conditioned medium = 65 ± 7; media with N^GNitro-L-arginine = 69 ± 12). Addition of rat amyloid β 1–42 (20 μg/ml, Calbiochem Co., San Diego, CA) and TNF-α (500 pmol/ml, Sigma, St. Louis, MO) evoked little neuronal necrosis (24.4% ± 1.1, 18.3% ± 0.3, respectively) suggesting that these proteins are unlikely to account for the microvessel-mediated neurotoxicity.

AD Microvessel Cytotoxicity Is Neurospecific

Although exposure to AD microvessels kills primary neuronal cultures and primary cerebellar granule neurons, the viability of seven non-neuronal cell types, including brain-derived glia, was unaffected (Figure 2). Other evidence that AD microvessel toxicity is neurospecific comes from experiments showing that the differentiated

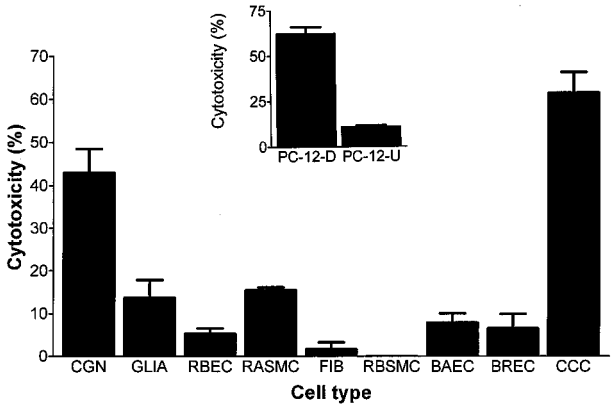

Figure 2. AD microvessel cytotoxicity is neurospecific. AD microvessels were co-cultured in 24-well plates with the following different cell types: primary rat cerebellar granule neurons (CGN), rat brain glial cells (GLIA), rat brain endothelial cells (RBEC), rat aorta smooth muscle cells (RASMC), rat fibroblasts (FIB), rat brain smooth muscle cells (RBSMC), bovine aorta endothelial cells (BAEC), bovine retinal endothelial cells (BREC), and primary rat cerebral cortical cultures(CCC). After 4 hours, 100 μl aliquots of media were assayed for LDH release. Inset: Undifferentiated (PC-12-U) and differentiated (PC-12-D) PC-12 cells were co-cultured with microvessels as above. Data are expressed as a percentage of total LDH release. Each bar represents the mean ± SE of three experiments performed in duplicate.

PC-12 neuronal cell line is killed in the presence of AD microvessels, but there is no cytotoxic effect on the same cell line when PC-12 cells are undifferentiated and therefore non-neuronal (Figure 2, inset).

Neuronal Cell Death Occurs by Either Apoptosis or Necrosis and Is Microvessel Dose- and Time-Dependent

The very short time required for cell killing (<4 hours), as assessed by the release of LDH, was consistent with necrosis. Because neurons can undergo either necrosis or apoptosis, depending on the intensity of the insult,[22] we examined the mechanism of cell death evoked by AD microvessels directly or by microvessel-conditioned media over a wide concentration range. The data show that AD microvessels can evoke either neuronal apoptosis or necrosis and the balance between these two pathways is reciprocal and dose-dependent. High concentrations of conditioned media from AD microvessels caused predominately necrosis, whereas apoptosis was more prominent at lower concentrations (Figure 3A). Co-culture with 10 μg of AD vessel protein caused a ninefold higher level of neuronal apoptosis than co-culture with 100 μg (Figure 3B). Ten μl of medium conditioned by exposure to AD microvessels required 24 hours to evoke a maximal apoptotic response in neurons (Figure 3C), whereas the time necessary for 50 μl of conditioned medium from AD vessels to elicit maximal neuronal apoptosis was only 9 hours (Figure 3D). The apoptotic response was less after exposure to 50 μl compared with 10 μl of AD-conditioned medium because of the greater necrotic response at the higher dose of conditioned medium. These data suggest that neurotoxic activity is dependent on both the amount of microvessel protein and the duration of medium conditioning.

Discussion

Because neuronal cell loss underlies the dementia of AD, identification of factors that cause lethal neuronal injury is central to understanding the pathogenesis of this disease and ultimately to developing effective therapies. Our present finding that co-culture of AD microvessels with neurons or addition of microvessel-conditioned medium to neurons causes neuronal cell death identifies the cerebral vasculature as a novel source of neurotoxic factors in the brains of AD patients. The potency of the vascular-derived neurotoxic factor, as well as the rapidity of the lethal effect on neurons could be consistent with the release from microvessels of nonspecific toxic molecules, such as reactive oxygen species. Indeed, when endothelial cells are activated or injured they produce both superoxide and hydroxyl radicals as well as nitric oxide.[23,24] However, the vascular-derived factor reported here only kills primary cortical neurons, primary cerebellar granule neurons, and the differentiated (ie, neuronal) PC-12 cell line. This factor released by microvessels does not kill non-neuronal cell types. The toxic factor is therefore not general but rather very neurospecific. It should be noted that our data heretofore cannot distinguish between a single neurotoxin or the actions of multiple factors in the pathogenesis of vascular-mediated neurotoxicity.

Attempts to understand the pathogenesis of AD have focused on the development of senile plaques and neurofibrillary tangles and how these lesions contribute to neuronal cell loss. In this study, we describe for the first time direct neuronal cell death mediated by isolated blood vessels from AD patients. Although the mechanisms by which this acute *in vitro* toxic response are related to the pathogenesis of the slowly progressing chronic disease AD are unclear, the implications of this observation for understanding and studying AD are broad and potentially exciting. While the cortex is richly vascularized, the proximity and organization of vessels and neurons *in vivo* is quite different from the *in vitro* co-culture model. Thus, the dilution of this factor, its rate of diffusion, or its capture by other extracellular molecules *in vivo* could be significant. Also, the presence of additional cell types *in vivo*, such as glia and microglia, could affect the spatial and temporal availability (eg, via metabolism) of the vascular-derived neurotoxic factor. Furthermore, because the cerebral endothelium is continuously exposed to potentially noxious elements and inflammatory mediators present in the blood, endothelial cell injury, which is presumably a cause of neurotoxin release, may occur slowly over a period of years.

Whereas the identity of this factor remains to be determined, we have ruled out several candidate neurotoxins including nitric oxide, TNF-α, and amyloid β. Because production of the neurotoxic factor is inhibited by cycloheximide and is heat and trypsin sensitive, it is most likely a protein. Preliminary studies using Centricon devices suggest a molecular weight between 10,000 and 50,000. Failure of the nitric oxide synthase inhibitor N^GNitro-L-arginine to block toxicity also supports the notion that nitric oxide does not mediate the cell death reported

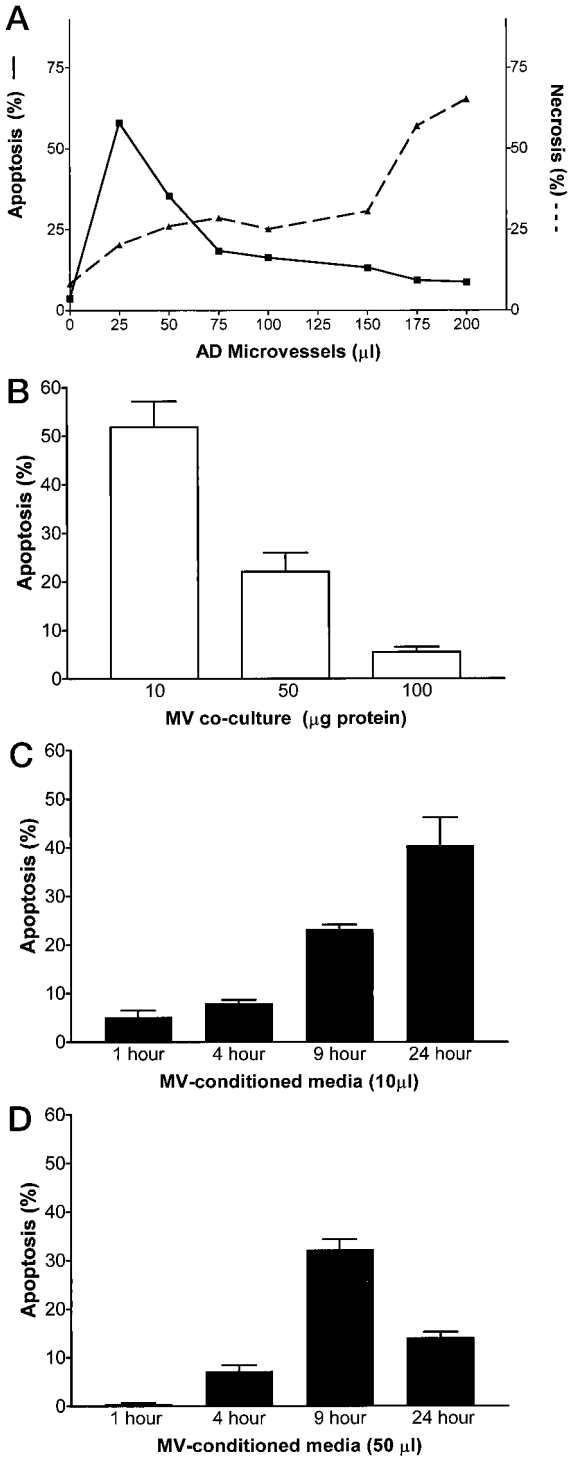

Figure 3. Apoptosis by AD microvessels is time and dose-dependent. For **A**, conditioned media (collected after 4 hours) from variable amounts of AD microvessels (25 to 200 μg protein) were added to primary cerebral cortical cultures. At 4 hours, an aliquot of culture medium was removed and assayed for LDH release (broken line). At 24 hours, the same cells were then solubilized and apoptosis (solid line) was determined by enzyme-linked immunosorbent assay measurement of nucleosomes as described in methods. Each point represents the average of duplicates. For **B**, varying amounts of AD microvessels were co-cultured with cerebral cortical cultures for 24 hours and apoptosis was quantified by enzyme-linked immunosorbent assay. In **C** and **D**, medium conditioned by AD microvessels for various times (1 to 24 hours) was centrifuged and 10 (**C**) or 50 μl (**D**) was added to cerebral cortical cultures, which were then incubated for 24 hours prior and apoptosis determined. Each bar represents the mean ± SE of two experiments performed in duplicate.

herein. Although amyloid-β and TNF-α, at the concentrations tested, can evoke apoptotic neuronal cell death at 24 hours (data not shown), the inability of either protein to cause significant neuronal injury at 4 hours, the relatively short time required for the microvessel toxin to kill neurons, suggests these proteins do not contribute to microvessel-mediated neurotoxicity. Whether the mediator is a known molecule or a previously unidentified species, our results demonstrate that vascular-derived neurotoxic factors are an important new paradigm of neuronal injury in AD.

Necrosis and apoptosis are two distinct mechanisms of cell death differing in their effects on cellular morphology and metabolism. Necrosis is usually evoked by intense insults and is characterized by cell swelling, membrane lysis, injury to cytoplasmic organelles, and release of cellular contents. The apoptotic cell death program is defined by cell shrinkage, membrane blebbing, nuclear pyknosis, chromatin condensation, and genomic fragmentation.[25] Although necrosis and apoptosis are distinct processes, they may also represent extremes of a cell death continuum that is dependent on the intensity and duration of the stimulus as well as the status of the target cell. Our results show that the endothelial-derived toxic factor evokes either necrosis or apoptosis depending on the microvessel dose. This finding is consistent with the data from experiments of others[22] using other neurotoxic agents, such as *N*-methyl-D-aspartate and nitric oxide, that also evoke both apoptotic and necrotic patterns of cell death.[22]

The present experiments are the first to identify the cerebral vasculature as a source of neurotoxic molecules. The data support a new paradigm, designated the END hypothesis (endothelium-mediated neurodegeneration), which postulates that AD brain microvessels produce soluble factors that can injure or kill neurons. These results highlight the cerebral microcirculation as a novel, unexplored source of neurotoxic factors in AD and a heretofore unrecognized target for therapeutic intervention.

Acknowledgments

We thank Drs. E. Brandt, R. E. Anderson, R. Floyd, and R. McEver for helpful discussions and comments, and Dr. R. Brumback (OU Diagnostic Center for AD) and Dr. B. Ghetti (Indiana University) for providing human brain tissue. Dr. Grammas is the recipient of the Alfred M. Shideler Professorship in Experimental Pathology.

References

1. Terry RD, Katzman R, Bick KL: Alzheimer Disease. New York, Raven Press, 1994
2. Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J: Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991, 349:704–706
3. Schellenberg GD, Bird TD, Wijsman EM, Orr HT, Anderson L, Nemens E, White JA, Bonnycastle L, Heston LL, Martin GM: Genetic linkage

evidence for a familial Alzheimer's disease locus on chromosome 14. Science 1992, 258:668–671
4. Levy-Lahad E, Wijsman EM, Nemens E, Anderson L, Goddard KAB, Weber JL, Bird TD, Schellenberg GD: A familial Alzheimer's disease locus on chromosome 1. Science 1995, 269:970–973
5. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD: Apolipoprotein E: High-activity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer's disease. Proc Natl Acad Sci USA 1993, 90:1977–1981
6. Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson L-A, Nilsson L, Persson G, Odén A, Svanbort A: 15-year longitudinal study of blood pressure and dementia. Lancet 1996, 347:1141–1145
7. Hofman A, Ott A, Breteler MMB, Bots ML, Slooter AJC, van Harskamp F, van Duijn CN, Van Broeckhoven C, Grobbee DE: Atherosclerosis, apolipoprotein E, and prevalence of dementia, and Alzheimer's disease in the Rotterdam study. Lancet 1997, 349:151–154
8. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR: Brain infarction and the clinical expression of Alzheimer disease. JAMA 1997, 277:813–817
9. Davies DC, Hardy JA: Blood-brain barrier in aging and Alzheimer's disease. Neurobiol Aging 1988, 9:46–48
10. Masters CL, Beyreuther K: The blood-brain barrier in Alzheimer's disease and normal aging. Neurobiol Aging 1988, 9:43–44
11. de la Torre JC, Mussivand T: Can disturbed brain microcirculation cause Alzheimer's disease? Neurol Res 1993, 15:146–153
12. Kalaria RN: Cerebral vessels in ageing and Alzheimer's disease. Pharmacol Ther 1996, 72:193–214
13. Kalaria RN, Harik SI: Reduced glucose transporter at the blood-brain barrier in cerebral cortex in Alzheimer's disease. J Neurochem 1989, 53:1083–1088
14. Grammas P, Roher AE, Ball MJ: Decreased α-adrenergic receptors at the blood-brain barrier in Alzheimer's disease. Alzheimer's Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies. Edited by K Iqbal, DRC McLachlan, B Winblad, HM Wisnieski. New York, John Wiley & Sons, Ltd, 1991, pp 129–136
15. Grammas P, Roher A, Ball MJ: Increased accumulation of cAMP in cerebral microvessels in Alzheimer's disease. Neurobiol Aging 1994, 15:113–116
16. Cashman RE, Grammas P: cAMP-dependent protein kinase in cerebral microvessels in aging and Alzheimer's disease. Mol Chem Neuropathol 1995, 26:247–258
17. Grammas P, Moore P, Botchlet T, Hanson-Painton O, Cooper DR, Ball MJ, Roher A: Cerebral microvessels in Alzheimer's have reduced protein kinase C activity. Neurobiol Aging 1995, 16:563–569
18. Dorheim MA, Tracey WR, Pollock JS, Grammas P: Nitric oxide is elevated in Alzheimer's brain microvessels. Biochem Biophys Res Commun 1994, 205:659–665
19. Pereira HA, Kumar P, Grammas P: Expression of CAP37, a novel inflammatory mediator in Alzheimer's disease. Neurobiol Aging 1996, 17:753–759
20. Grammas P, Moore P, Weigel PH: Production of neurotoxic factors by brain endothelium in AD. Ann NY Acad Sci 1997, 826:47–55
21. Greene LA, Tischler AS: Establishment of a noradreneryic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA 1976, 73:2424–2428
22. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA: Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell culture. Proc Natl Acad Sci USA 1995, 92:7162
23. Nakazano K, Watanabe N, Matsuno K, Sasaki J, Jato T, Inoue M: Does superoxide underlie the pathogenesis of hypertension. Proc Natl Acad Sci USA 1991, 88:10045–10048
24. Kumar M, Liu G-J, Floyd RA, Grammas P: Anoxic injury of endothelial cells increases production of nitric oxide and hydroxyl radicals. Biochem Biophys Res Commun 1996, 219:497–501
25. Majno G, Joris I: Apoptosis, oncosis, and necrosis: an overview of cell death. Am J Pathol 1995, 146:3–15